EXHIBIT 45

B = Bio-11-dUTP

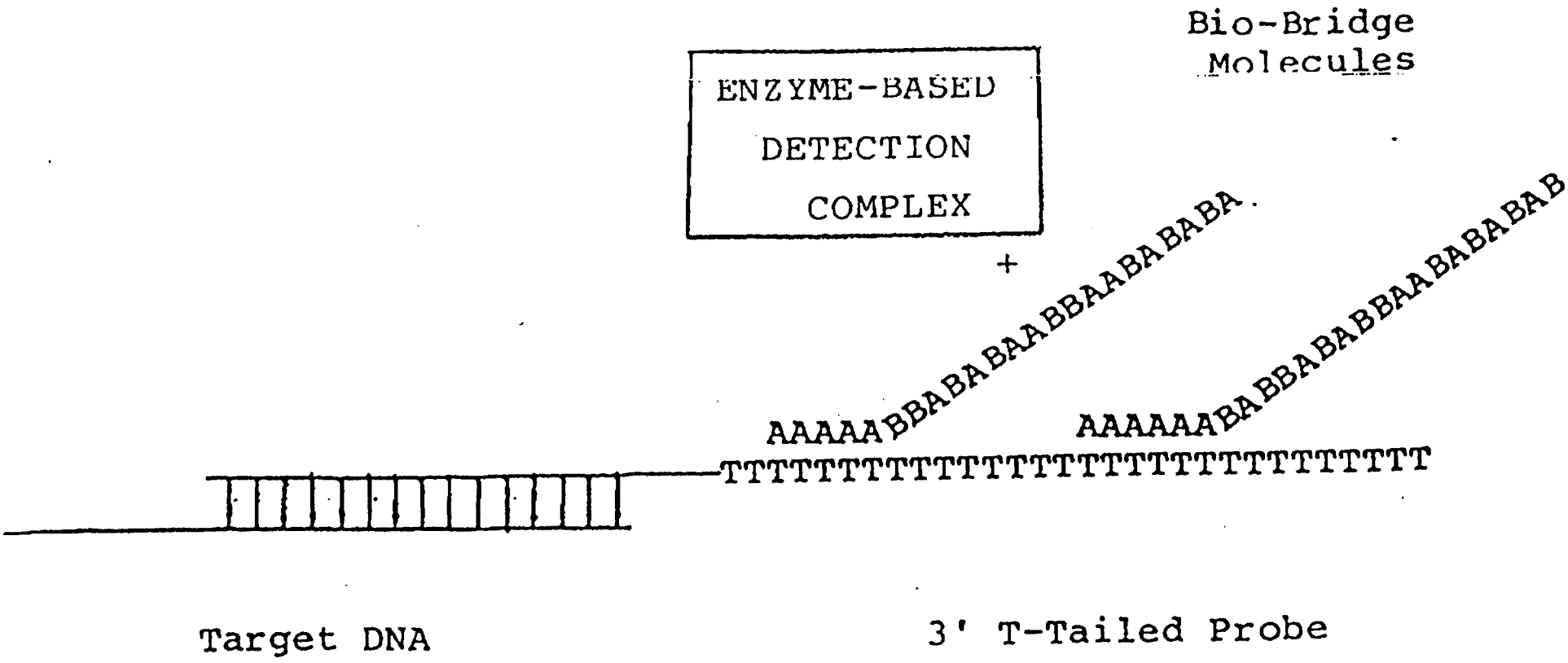
Bio-Bridge
Molecules
ENZYME-BASED
DETECTION
COMPLEX
+
AAAAABBABABAABBAABABABA
AAAAAABABBABABBAABABABAB
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
Target DNA
3' T-Tailed Probe

Figure 2: **Indirect Biotin Labeling of DNA Probes.**

DNA probes that are completely free of radioactive and modified nucleotides can be used for specific and sensitive detection of target DNA.

Such DNA probes are synthesized and used as described in the following procedure:

1. DNA to be used as probe is treated with DNase or restriction endonuclease to generate 3'-OH termini.

2. Homopolymeric terminal additions to the 3'-OH termini are synthesized using terminal deoxynucleotide transferase (TdT) and either TTP or dATP (TTP terminal addition is shown here).

3. The terminally extended probe DNA is hybridized to fixed target DNAs and appropriate washes are carried out.

4. The hybridized filter is exposed briefly to the bridging (labeling) molecule, Bio-Bridge™A, a Biotin-11-dUTP-modified oligo dA (or to Bio-Bridge™T when polydA terminal labeled DNA is used as probe).

5. The bridging (labeling) molecule serves to link the hybridized probe DNA to a biotin-based detection system (such as Detek™ shown here).

Figure 3: Identification and Use of Bridging Molecules

In order to identify effective bridging molecules, dot blots of various terminally extended DNAs were exposed to different bridging molecules and then the sensitivity and specificity of detection with the bridging molecules was examined using Detek I-hrp or Detek I-acp.

A. - C. Dot blots of TTP/Biotin-dUTP terminal-labeled DNA (left lanes) and TTP terminal-labeled DNA (right lanes) detected with Detek I-hrp after

A. NO BRIDGING;

B. BRIDGING with BIO-BRIDGE A (dA_x:dA_y, Bio-dU_z; or

C. BRIDGING with BIO-BRIDGE T (dT_x:dT_y, Bio-dU_z).

D. - E. Dot blots of dATP/Biotin-dUTP terminal-labeled DNA (left lanes) and dATP terminal-labeled DNA (right lanes) detected with Detek I-acp after

D. NO BRIDGING;

E. BRIDGING with BIO-BRIDGE A (dA_x:dA_y, Bio-dU_z); or

F. BRIDGING with BIO-BRIDGE T (dT_x:dT_y, Bio-dU_z).

Figure 4: Southern Blot Hybridization and Indirect Detection.

I. **Sensitivity of Detection:** Southern transfers of dilutions of Bam H-I digested pBK322-B$^+$ Pst(4.4kb) hybridized to A. nick translated probe DNA; B. TTP/Biotin-dUTP terminal-labeled probe DNA; and C. TTP terminal labeled probe DNA and bridging with Bio-Bridge A. Detection was accomplished with Detek I-acp.

II. **Specificity of Detection:** Bam HI digests of mouse DNA and human placental DNA were subjected to electrophoresis, Southern transfer, hybridization to a TTP terminal-labeled probe for 28S rDNA, and bridging with Bio-Bridge A. Specificity is shown by the identification of the appropriate hybrid bands in mouse DNA (6.8, 5.3, 2.9 and 1.8kb) and in human DNA (6.8, 5.3 and 1.8 kb).

Figure 5: **Double Detection Using Direct and Indirect Biotin Labeling.**

DNA dot blots of TTP/Biotin-dUTP (left lanes) and TTP (right lanes) terminal labeled DNAs were used to demonstrate that two detections could be achieved on a single dot blot or Southern transfer.

A. Detection with Detek I-hrp with no other treatment.

B. Addition of Bio-Bridge A followed by detection with Detek I-acp.

C. Detection with Detek I-hrp followed by addition of Bio-Bridge A and subsequent detection with Detek I-acp.

Figure 6: Double Detection on Southern Transfers.

A Southern transfer of Bam H-I digested pBR322 B^+ globin Pst (4.4kb) was hybridized with biotin-labeled nick translated pBR322 and simultaneously with the 1.8kb Bam HI fragment of B globin DNA that had been TTP terminal-labeled. After the hybridized filter was washed, nick translated biotin-labeled probe was detected with Detek I-hrp resulting in brown colored bands at 4.9kb and 1.3kb, the only fragments containing pBR322 sequences (see Polaroid photograph). Following detection of directly biotinylated probe, the filter was washed and exposed to Bio-Bridge A. The sites of hybridization of the 1.8kb fragment were determined using Detek I-acp which gave violet-colored bands in the appropriate location.